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UNITED STATES DEPARTMENT OF AGRICULTURE

BUREAU OF ENTOMOLOGY

FOREST INSECT INVESTIGATIONS

THE CONCENTRATION OF CERTAIN SUGARS IN THE BARK OF THE WESTERN YELLOW PINE AS RELATED TO WESTERN PINE BEETLE ATTRACTION

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SUMMARY

The concentration of certain sugars in the bark, especially the inner bark, of western yellow pine was investigated as a part of the study on the attraction of the western pine beetle. The following are the results, to date, of this investigation:

1. Short logs which had been cut for any considerable length of time were found to be an uncertain source of material, due to changes in the sugar concentrations and in moisture content.

2. In recently-felled trees, before attack, there is evidence of an increase in levulose and a decrease in sucrose.

3. When these trap trees are attacked by D. brevicomis the reducing sugars in the inner bark, especially levulose, increase, and the sucrose decreases rapidly. This is followed by a decrease in all sugars, which is especially rapid close to the beetle galleries. The decrease is probably the result of abnormally high cell respiration and of fermentation due to the microörganisms introduced by the beetle. At the time the larvae leave the inner bark the sugar concentrations have fallen to lower values than exist in the outer bark, to which they migrate.

4. Following the loss of sugars in the attacked logs there is an increase in the moisture. This is evidently due to the water formed in the process of respiration.

5. In comparing slow- and fast-growing trees it was found that the average concentration of levulose is higher in slow-growing trees than in fast, and the sucrose concentration and moisture content are lower. The pH value is also lower in the slow growing trees; i.e., the acidity is slightly greater.

6. Slow-growing trees and felled trees are both attractive to D. brevicomis. Since it was found that both of these classes have high levulose and low sucrose concentrations, the theory is advanced that an attractive substance may be formed by a change associated with, or occurring under similar conditions to, the hydrolysis of sucrose.

7. There seems to be but little relation between Dunning's tree classes and sugar concentration. This may be due to the small number of trees studied.

8. No relationship was found between tree diameter and any of the values here determined, which again may be due to small number of trees.

9. Dextrose increases in concentration during the season studied (July to October). Sucrose may show an early seasonal increase but varies little during the latter part. Levulose shows no significant seasonal change.

10. A preliminary test on an attacked standing tree showed lower sugar concentrations in the early stages of attack. This change is in the opposite direction from that found in trap logs, but there is not sufficient evidence from which to draw any conclusions.

11. The sugar concentrations at the end of the season in a tree seriously injured by fire were similar to those of the other trees at the time of the fire, and somewhat lower than the average concentrations at the time of determination.

12. It was demonstrated that studies such as these must be conducted in a laboratory very close to the trees being studied, to reduce to a minimum the deterioration of samples.

The suggestion is made that further work of similar nature should be done, especially to study:

1. The effect of types of injury known to increase the attractiveness of the trees to *D. brevicomis*, such as drought, fire injury and defoliation.

2. The changes in attacked standing trees as they were studied this year on trap logs.

3. The variation in pH on trees of different types and under different conditions.

THE CONCENTRATION OF CERTAIN SUGARS IN THE BARK OF THE WESTERN YELLOW PINE, AS RELATED TO WESTERN PINE BEETLE ATTRACTION

Preliminary Studies

I. Introduction

The studies reported in this paper were undertaken as a part of the more extensive studies being conducted by the Bureau of Entomology under the direction of Dr. F.C. Craighead and Mr. J.M. Miller on the relation of the western pine beetle, Dendroctonus brevicomis Lec., to its chief host tree, the western yellow pine, Pinus ponderosa Laws.

Tree selection studies by Person (15) show that there is a relation between amount of loss from D. brevicomis and the growth rate of the trees. He also found that diameter and tree class (Dunning's) could be correlated with the amount of loss. Person, Mirov (10) and others have shown that the inner bark is the most attractive part of the tree, and that fermented inner bark is especially attractive. Because of the importance of inner bark, both fresh and fermented, in the attraction of D. brevicomis it was thought advisable to concentrate further work on the study of this tissue. The sugars are the common substances used up either in fermentation by means of microorganisms or by anaerobic respiration of the tree, which might result from abnormal conditions such as drought, fire injury etc. Consequently it was decided that a study of the concentration of the different sugars of the inner bark would be a logical starting-place for chemico-physiological studies on attraction.

The studies included in this report were conducted to determine the normal concentration of the sugars--levulose, dextrose and sucrose--in the inner bark of western yellow pine; and also to determine whether variations from the normal could be used as an index of attractiveness. It was recognized that a change in the quantity of sugar would not directly affect the attractiveness of a tree; but it was believed that injury, drought or other conditions--which do cause a change in attractiveness--might also result in the production of compounds, such as esters and aldehydes, some of which might well be the attractive substances

The writer wishes to thank Professor W.V. Cruess of the Fruit Products Laboratory, University of California, for the use of special equipment and facilities of his laboratory. He is indebted to Dr. S.H. Cameron, of the Division of Subtropical Horticulture of the same university, for reading the manuscript and for helpful suggestions. He also wishes to thank Mr. H.L. Person for many suggestions and valuable criticism throughout the work.

II Methods

Some preliminary work was done in Berkeley to develop technic applicable to the material to be used in the sugar determinations and to determine what equipment would be needed under field conditions.

The field work here reported was started in the latter part of May, 1929, at the field laboratory of the Bureau of Entomology. This laboratory is located near Buck Creek Ranger Station, on the west slope of the North Warner Mountains, at an elevation of about 5,000 feet, on the Modoc National Forest, California. The records of temperature and relative humidity were taken during the field season with a hygrothermograph. They are summarized in Plate I.

A. Tree Records

Records were taken on all trees studied, with the exception of those used for preliminary work. These records were necessary for correlating the concentration of the various sugars, moisture content and pH with the growth rate, tree class, diameter and other factors included in the records. In these studies records were taken under the following headings:

1. Rate of growth
    a. Width of 1928 ring (in mm.)
    b. Rings in last half-inch
    c. " " " inch
    d. " " each inch, cambium to center (if tree were felled)

2. Crown
    a. Length
    b. Width
    c. Terminal growth
    d. Foliage condition

3. Bark
    a. Outer bark
        1. Color
        2. Thickness
        3. Depth of cracks
        4. Width of plates
        5. Relative number of resin pits
    b. Inner bark
        1. Thickness
        2. Apparent moisture content

4. Diameter

5. Age

6. Height

7. Dunning's tree class

3. Location
   - a. Geographic
   - b. Topographic exposure
   - c. Position with respect to other trees
   - d. Soil

D. Preparation of Sample

Nearly all the analyses here reported were conducted on the inner bark of the western yellow pine. The term "inner bark" is here used to designate the soft, nearly white layer of living tissue between the fascicular cambium on the inside and the phellogen or cork cambium on the outside. This is the tissue in which the attacking parent adult beetles make their egg galleries and lay their eggs, and in which the larvae feed until about one-quarter grown. The first step in the collection of the samples was the cutting off of the outer bark down through the last dead scales to where the tissue appeared to be homogeneous. The inner bark was then removed down to the cambium, with as little bruising as possible, and immediately transferred to a glass jar and tightly closed. If any considerable quantity of this material was needed, the outer bark was taken from only a small part at a time, and that removed and quickly placed in the jar before the outer bark was removed from another part. This was done to decrease the error due to evaporation and oxidation as far as possible. The sample was then taken to the laboratory and immediately run through a hand grinder, once with a coarse cutter and twice with a "nut butter" cutter. A given quantity was then quickly weighed out and placed in a volumetric flask; distilled water was added and the flask placed in a steam bath.[1] The remainder of the sample was replaced in the tightly-closed fruit jar until moisture determinations could be run; these were run as soon as possible.

Whether the samples were taken from short blocks, logs in the field or living trees, the methods used were the same. This is especially important in the securing of comparable results.

When the outer bark, i.e., the dry dead layer outside of the phellogen, was used for analysis the treatment was similar, except that rapid handling was not so important because the tissue was already dead and consequently not subject to enzymic change, and was already in equilibrium with the air so far as moisture content was concerned.

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[1] The bath consisted of a square sheet metal box containing about an inch of water in the bottom, automatically held at that height by a regulated inflow and outflow and kept boiling vigorously by a gasoline stove. There were four holes in the top of the bath through which flasks could be inserted to just above the water level. The lids were then replaced, the necks of the flasks fitting through holes in the lids. The boiling point of water at the laboratory was about 95°C. (203° F.), due to elevation.

The flask containing tissue and distilled water was allowed to remain on the steam bath for 2½ hours. This time was used because it was found in preliminary experiments that it gave the maximum value for sugars without extracting gummy substances which slowed down filtration. When the sample was removed from the steam bath it was cooled in cold running water, and distilled water was added to make a given volume. It was then allowed to stand to attain uniformity of sugar concentrations between the solid portions saturated with water and the solution. The usual method was to employ either a 50-g. sample and "make up to" 500 cc. or else a 100-g. sample and make up to 1,000 cc.; i.e., so that each 10 cc. of solution corresponded to one gram of the original sample. This 10-to-1 relation was never varied much, and when it was varied at all a correction was made in the calculations. After the sample had reached equilibrium it was filtered. At the first of the season a coarse filter paper was used for this filtration; but later it was found that much time could be saved, and consequently evaporation during filtration decreased and a greater recovery of solution obtained, by filtering first through a copper wire screen (18-mesh) and then through a funnel containing a cotton plug.

At this point the pH of the solution was determined. The method used was that given by Clark (5), using his color charts and indicator solutions of the required strengths. The absolute accuracy of this method is probably not very great and its sensitivity not all that could be desired; but it was found that checks could be obtained at widely different times which were never more than 0.1 pH division apart and usually on the same tenth. This solution was used instead of the tissue itself because the charts were made up for comparison in a test tube.

The next step was clarification of the solution. This was done by Horne's (7) method. This consists of the addition of dry basic lead acetate, which precipitates the proteins, gums etc. This powder is carefully added until all the substances it precipitates are removed but no great excess of lead is present. The insoluble lead compounds are then filtered out and the necessary slight excess of lead similarly precipitated by careful addition of dry sodium oxalate, giving insoluble lead oxalate, which is removed by another filtration. The solution is then ready for the sugar determinations to be made.

C. Reducing Sugar Determinations

1. Choice of method

There are many different methods of sugar determination in use, but they are all empirical and were nearly all designed for use on commercial sugars where the amount of sugar is great in relation to impurities. Also most of these methods require the use of facilities, such as electricity or vacuum, not available at the field laboratory where this work had to be done to eliminate excessive deterioration of samples. Other methods require the use of expensive apparatus. The basic method chosen was that of Shaffer and Hartman (16).

This is essentially a method using Fehling's solution and the technique of the Munson-Walker (12) method, modified for volumetric determination by means of iodometry. A value is obtained which is multiplied by a factor to give milligrams of copper reduced. The product is used in the "Invert sugar + sucrose = 0.4 grams" column of the Munson-Walker tables to find "milligrams of sugar" in the quantity of solution taken. The factor given by the original authors (6.36) was verified for field laboratory conditions, using a known quantity of hydrolyzed C.P. sucrose. It was expected that this factor would not exactly correspond with the original, due to differences in conditions, chief among which is the difference in the boiling point of water previously referred to; but the published factor was obtained.

D. Total Sugar Determinations

The sugar solution was also hydrolyzed by means of 5 cc. of concentrated HCl added to 40 cc. of sugar solution on the above-described steam bath, so regulated as to raise the temperature to 68° C. in ten minutes. The figure here obtained was converted into "mg sugar" by means of the "invert sugar" column in the Munson-Walker (12) tables.

The difference between the above results was multiplied by 0.95 and taken as the sucrose value. It is clearly recognized that this method does not give actual percentage of sucrose. However, it does give a value which, after the technique is developed, can be duplicated with a fair degree of accuracy and which, in view of the small amount of exact knowledge available, is just as likely to be of importance as the more accurate value for sucrose obtained by the more-time-consuming enzymic process. The reducing sugar value plus the sucrose value was taken as the total sugars. This result of course contains the inaccuracies of the basic determinations.

E. Levulose Determinations

The separation of the reducing sugars was accomplished by determining the levulose by means of Ost's solution, using a water bath at about 48.5° C. for 2½ hours (13), followed by a Shaffer-Hartman determination of the reduced copper. The method is claimed by the original author to give little if any other sugar. The difference between reducing sugar and levulose values is called dextrose, though it is recognized that it includes one-half of the maltose present and any other reducing substances not removed by the clarification method, as well as dextrose.

Though the values here obtained are admittedly somewhat arbitrary, there is no known method of sugar determinations for material the composition of which is as complex and as little known as this, which would not introduce sources of error. It seemed that this method was as accurate as would be significant in a piece of preliminary work without sacrificing any more time or money than was necessary. A much larger number of determinations than it was possible to make, even by these methods, would have been of great assistance in properly interpreting the results.

B. Starch Determinations

Starch was not regularly estimated quantitatively during the field season. It has been shown by S.H. Cameron of the Division of Subtropical Horticulture, University of California, in an unpublished report, that in the case of citrus and pear trees the starch was not all determined unless the whole sample was ground to a size to pass through a 100-mesh screen. Apparently the recovery was very irregular unless all cells were broken. He found the bark more difficult to reduce than the much harder wood. In order to attain this degree of fineness he had to use a ball mill. This could not be done in the field, due to lack of electricity necessary for the twelve-hour continuous operation of the mill, but was done on a few samples after returning to Berkeley. However, a very rough quantitative determination was run somewhat incidentally by observation of the depth of blue color produced by a given quantity of iodine.

Dr. Cameron is still working on the method of starch determination which I used on the samples run in Berkeley. However, the procedure now employed seems to be more reliable than the official methods (8) for all samples of wood and bark that he has used. Briefly, it is a method making use of extraction with 95% alcohol, followed by treatment in the ball mill for fine grinding and agitation during the enzymic (saliva) hydrolysis, which is followed by an acid hydrolysis.

C. Moisture Determination

No official method of moisture determination is given for plants in the "Method of Analysis" of the Association of Official Agricultural Chemists (8). The nearest approach to similar material for which methods are given is for "Fruits and Fruit Products". This official method was used in Berkeley. It requires drying at a temperature of 70° C. under vacuum. As this was impossible in the field, the boiling water oven was used. This oven operated at a temperature of 94° C. It was found in preliminary tests that a drying time of three hours was necessary to obtain a constant weight. The apparatus and precautions were similar to those described for the water oven in the above-mentioned book.

D. Expression of Results

The results are recorded primarily as percentage by weight on the wet or fresh basis. This can easily be obtained because the values derived from the Munson-Walker (12) tables are in milligrams for the amount of solution used; thus the calculation is:

$$\frac{\text{mg sugar x capacity of volumetric flask in cc. x 100}}{\text{1000 x weight of sample taken x no. cc. sugar solution taken}} = \text{\% sugar}$$

The number of cubic centimeters of sugar solution had to be varied in order to keep the amount of sugar in the solution between the limits of 50 and 300 mg., within which range the method is most accurate. Since the weight of sample taken was numerically one-tenth of the capacity of the flask, the second fraction in the expression becomes 10 and the whole expression cancels down to:

$$\frac{\text{mg sugar}}{\text{50 cc. sugar solution}} = \%\ \text{sugar}$$

This value for the fresh basis was also multiplied by the ratio $\frac{100}{\%\ \text{dry wt.}}$ to give percentage sugars on the dry basis. The percentage on the fresh basis is used in all calculations and graphs because, as shown by Mason and Maskell (9), the moisture content of the samples does not change as much as the reserve carbohydrates do, the latter change having a larger effect on the basis for the dry weight. The concentrations on the dry basis are included in the tables, however, for comparison with the large amount of work done on this basis. The "Residual Dry Weight" basis advanced by Mason and Maskell and based on dry weight less reserve carbohydrates, could not be used here, due to lack of determination of reserve carbohydrates. In any case this method of calculation has not yet attained wide use.

III. Characterization of Sugars

In connection with this work a brief characterization of the chemical and physiological properties of the sugars under consideration may be of value. This is largely adapted from Armstrong (1), to whom the reader is referred for a more complete chemical treatment of this phase of the subject.

Levulose is also known as laevulose and d-fructose, and less exactly as fruit sugar. It has a large number of isomeric forms, but the partial structural formulae of the forms in which most of it probably exists in solution in water are (3):

$$CH_2(OH)\ .\ \underbrace{CH\ .\ CH(OH)\ .\ CH(OH)\ .\ C(OH)}_{O}\ .\ CH_2(OH)$$

$$\overbrace{CH_2\ .\ CH(OH)\ .\ CH(OH)\ .\ C(OH)\ .\ C}^{O}\ .\ CH_2(OH)$$

These are known as the butylene and amylene oxide formulae respectively, and are probably both in equilibrium with the more active keto form,

$$\begin{array}{ccccccccccc} & & H & & H & & OH & & & & \\ CH_2(OH) & . & C & . & C & . & C & . & CO & . & CH_2(OH) \\ & & OH & & OH & & H & & & & \end{array}$$

Levulose crystallizes with the greatest difficulty of any of the sugars under consideration, and is the sweetest of the three. It has been shown by Castellani (4) and others that levulose or dextrose can be used selectively by certain microörganisms, and that in some similar sugars certain microörganisms are able to use selectively one of two or more isomers which cannot be separated by chemical methods.

Dextrose is also known as glucose and grape sugar. A mixture of equal quantities of dextrose and levulose is known as invert sugar because such a mixture is formed by the inversion or hydrolysis of sucrose. Dextrose probably exists chiefly in the ring form:

$CH(OH) \cdot CH(OH) \cdot CH(OH) \cdot CH \cdot CH(OH) \cdot CH_2(OH)$

O

but is also present to some extent in the aldehyde form:

$$CH_2(OH) \cdot CH(OH) \cdot CH(OH) \cdot CH(OH) \cdot CH(OH) \cdot CHO$$

Dextrose does not form large clear crystals as does sucrose, but may be crystallized as a powder. It is not as sweet as either levulose or sucrose.

Sucrose is also known as saccharose. The cane and beet sugars of commerce are both nearly pure sucrose. It is intermediate in sweetness between the other two and probably exists in solution almost entirely in one form. It does not show either aldehydic or ketonic properties and consequently does not reduce Fehling's solution--i.e., is not a reducing sugar. It is composed of one molecule of levulose, probably of the amylene oxide form, united to one molecule of dextrose with the loss of one molecule of water. Its structure is:

$CH_2(OH) \cdot CH(OH) \cdot CH \cdot CH(OH) \cdot CH(OH) \cdot CH$ (dextrose part)

O

O

O

$CH_2 \cdot CH(OH) \cdot CH(OH) \cdot CH(OH) \cdot C \cdot CH_2OH$ (levulose part)

Since sucrose is made up of units identical with dextrose and levulose, it is probable that the latter are formed first in photosynthesis, and that the two units are combined with the loss of one molecule of water to give sucrose. During photosynthesis in some plants the sucrose increases in concentration in the leaves, whereas the reducing sugars do not (5); but this may be because the rate of synthesis is approximately equal to the photosynthetic rate (6).

Sucrose may be broken down into dextrose and levulose either by acid hydrolysis or by the enzyme invertase (also known as saccharose sucrase and invertin). In the case under consideration the hydrolysis is made possible primarily by the presence of the enzyme. It has been found in a number of cases that organisms which do not produce this enzyme are entirely unable to utilize sucrose; so it must be assumed that none of the organisms present, the tree itself, the yeasts and blue stain, or the beetle, is able to use sucrose without first breaking it down to dextrose and levulose.

Starch is also present, as shown by qualitative tests with iodine and by a few determinations made after returning to Berkeley. The starch could not be directly connected with actual attraction because it is not volatile and is insoluble in the cell sap, acting only by first changing over into the simpler sugars, which were determined.

Starch, in changing into sugars, passes first through a number of dextrins and then into maltose, a sugar of the same block formula as sucrose but with different atomic configuration, so as to make it partially oxidized by Fehling's solution, and so one-half of it is recorded as reducing sugars. Maltose is probably present in only a small concentration and is soon changed to dextrose. All the changes during the conversion of starch to dextrose are hydrolyses. The same set of changes apparently go on in a reverse direction when starch is formed. Since starch is insoluble it can serve only as a storage food, which must be converted back into sugars to be transported or utilized by the plant, by the microorganisms or by the beetle. In order to cause this hydrolysis of starch at ordinary temperature the enzyme diastase is required. The enzyme must be produced by the normal cells of the phloem of the tree or else it could not be formed and used in the normal tree. Little can be said as to the possibility of the production of additional diastase by the yeasts present after beetle attack, by the "blue stain" or by the beetle itself with or without symbionts until more work has been done.

IV. Deterioration Studies

After the technique of the determinations had been fairly well standardized, the next point was to find what conditions had to be complied with in order to obtain samples as nearly as possible as they exist in the living tree. The first experiments of this group were conducted to find out how rapidly the sugar content of blocks about one foot long changed after the tree was felled. The blocks were placed in the shade of a tree near the laboratory. The significant results are given in Table I. A fairly rapid and rather inconsistent change in values may be observed from these and other experiments not sufficiently systematic to report in tables. There was also a considerable amount of drying, sufficient to make the phloem of the slower-growing tree very difficult to remove. For these reasons the use of small blocks for obtaining samples was abandoned.

V. Changes in sugar concentration and other values, etc., after felling and subsequent attack

Experiments were next started to determine the changes which take place in a log when the tree is felled and trimmed, with and without subsequent attack. These experiments were continued through the remainder of the field season.

The first samples used in this study were taken from the upper part of the log of tree #2 (see Table XIII for tree description), which had been left in the field and attacked by D. brevicomis. On the sides of the log the egg stage had been reached, but no attacks had as yet been made on the top. The tree was felled June 12 in a medium cool location, and the attacked sample taken July 2, twenty days later. The initial values are again included for comparison. The conditions on the top of the log vary from those on the side, due to the difference in temperature conditions as well as the absence of an attack. The results are given in Table II. It was planned to make a final determination on this log after the larvae left the phloem also, but it was burned in the Sugar Hill fire.

Since the values given in the table fit in quite well with similar stages in the logs more thoroughly studied, they need not be discussed separately.

Later stages of the changes caused by attack on trap logs were obtained by determinations on Log #0. The tree was cut May 27 and probably attacked early in June. On July 7 samples were taken from the sides of the log where the larvae were leaving the old blue-stained phloem, and from the top, to which the attacks had been more recently extended. This log was also destroyed by fire, but at this time the sides had probably nearly ceased to change in sugar concentration. The results are given in Table III, and discussion of them is covered with that of the other attacked logs.

The changes taking place after felling and after attack of the trap logs were further studied by following given trees from their initial value to the end of the season. This was first done on Tree #5, on which samples were taken from opposite sides of the tree on July 25. Four days later the tree was felled and two more samples were taken. Other samples were taken from time to time throughout the field season. After the attack had progressed sufficiently to make available large enough quantities of attacked phloem, samples were run on the attacked portions also. The attacked portion of the phloem had to be arbitrarily separated, and in this study all parts of the phloem within three-quarters of an inch longitudinally or half an inch tangentially of a gallery were assumed to be attacked, as was also any portion completely cut off by galleries not over 2" apart. This separation does not include all the tissue affected by the presence of the beetles and the microorganisms associated with it, as shown by the changes in the unattacked portion, but it was made with considerable care and fair uniformity, and so probably does as well as any other arbitrary separation. The results are given on a wet basis and also calculated over to the dry basis in Table IV. Since the moisture content is so nearly constant, only the results on the wet basis are graphically presented. These are divided into two graphs, however, in order to prevent confusion. Plate 2 shows in solid lines the changes up to the time when an amount of the attacked portion sufficient for analysis was available, and after that in dotted lines the changes in the attacked portion. Plate 3 shows the same early changes, but continues with the unattacked portion as long as sufficient quantities for analysis are available. At the time the plates were prepared the use of the term "inner bark" had not been decided upon. Consequently it is referred to as "living bark" and "phloem" in the plates.

Tree #6 was a slow-growing tree in an open stand on the top of a dry ridge. When it was felled the log had no natural shade but was covered with its own branches. This did not nearly make up for the differences in temperature between Tree #6 and Tree #5, as Tree #5 was in a creek bottom in a dense thicket of incense cedar, and even though left without artificial covering did not receive direct sunlight at any time of the day. Another difference in conditions was that #6 was felled nine days later than #5 and consequently right at the height of the flight period of the first summer brood of D. brevicomis. The two logs could not have been started at the same time and still get a sufficient number of initial and early points on both. The results on Log #6 are given in Plate 4 and Table V.

At a later date a third log, #7, was similarly treated, but, due to the approach of cool weather and lack of beetles, it was not attacked. It was in a rather open location but on a northeast slope and so not in as warm a position as Tree #6, though warmer than Tree #5. It was lightly covered with brush at first but later, as the weather became cooler, it was uncovered. The results are shown in Table VI and Plate 5.

Discussion of changes in trap logs after felling and subsequent attack

The first trees to be considered are #5 (see Table IV and Plates 2 and 3) and #6 (see Table V and Plate 4). In both, the most outstanding characteristic of the changes observed is the increase in reducing sugars, particularly levulose, and decrease in sucrose, undoubtedly caused by its hydrolysis over to the reducing sugars. The equation for the reaction is as follows:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} = \underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Levulose}}{C_6H_{12}O_6}$$

This reaction would be catalyzed by the enzyme invertase which is present in the plant tissue and which would probably also be introduced by microorganisms carried by the beetles, whether they were yeasts, or molds such as "blue stain". It would not be produced to any considerable extent by the beetles themselves, but the physiological effect of their work on the cells of the tree might easily be to produce or release this enzyme. The above equation calls for an equal increase of both sugars; but the greater increase in levulose than in dextrose which is given by the experimental results might be explained by a greater use of the latter in cell respiration, or by the microorganisms, or by the conversion of some of the dextrose to levulose by the plant or microorganisms. This latter change has been produced artificially.[8]

The total sugars increase more slowly in the unattacked portion, and then all the sugars slowly decline. This decline is so slow—as shown by Log #5, where such a comparison is possible--that the concentration was higher at the last time at which the unattacked portion was determinable than in the attacked portion, where a much higher peak had been reached. The changes in the attacked portion are, as would naturally be expected, more rapid and violent. Total sugar concentrations as high as ten per cent on the wet basis, or nearly 24 per cent on the dry basis, are recorded on Log #5, from which the values drop sharply to one-fourth of that amount in about a week and finally to less than one-half of one per cent, one-twentieth of the maximum value. There is no reasonable doubt of the significance of these changes. The difference in rate of change due to temperature was not fully realized at the time, nor was the previously-mentioned fact that Tree #6 was not felled until the flight of the first summer brood had started instead of about a week before the start, as was #5. Little change had occurred in #5 during the initial periods, so samples on #6 were not taken with sufficient frequency. However, it may be seen that if the numbers on the ordinate or time axis in Plate 4 are multiplied by a factor of about 2.2, they will correspond to a surprising degree with similar points in Plate 2 (Log #5). The high points of Plate 2 are not found in Plate 4 because no samples were taken at the proper time.

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[8] See Armstrong, pp. 57-59

It will be seen that the time required for the insects to reach a given stage also varies greatly between the two logs, but agrees fairly closely with the same conditions in the changes in sugar concentration. A point which may be of great importance is that the sugar concentrations have decreased greatly—to about one-third that in the normal living tree—by the time the larvae hatch. It may be seen that the values obtained on the two preliminary logs, #2 and #0, fit in quite well if put on either Plate 2 (Log #5) or Plate 3 (Log #6) at the point corresponding to the same stage of beetle development.

Some other points of interest may be enumerated as follows:

1. It will be noticed in Plate 2 that the first attacked sample shows a sharp rise in comparison to the previous sample from an unattacked portion. This rise occurs most markedly in dextrose. This is just what might be expected if the increase in total sugars is due to the hydrolysis of starch, which gives no levulose but does give first maltose (which is partially recorded as dextrose) and then further hydrolysis gives two molecules of dextrose for each molecule of maltose.

2. Levulose shows a quicker rise before attack but finally after attack decreases practically to zero. This might be considered to indicate its greater importance and will be mentioned later in another connection.

3. If the total sugars present at the maximum point found are lost by means of respiration, the equations for reducing sugars and sucrose respectively would be:

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O$$
$$C_{12}H_{22}O_{11} + 12\ O_2 = 12\ CO_2 + 11\ H_2O$$

If the sugars shown to be lost were broken down by the above equations, an amount of moisture would be formed equal to 5.8% of the original net weight. This may account for the comparatively small amount of loss in moisture of the log, since the evaporation is about equal to the production of water by respiration. It may be seen from Table IV that the moisture content at first decreased but toward the last, at about the time the sugar disappeared, it increased again. In fact, a net increase of 10% between Samples #11 and #15 was noted. These data are not at variance with the statement that an increase of 5.8% moisture might be due to the respiration of the sugars present on a given day, because both hydrolysis and respiration are continuous processes, and the amount of sugars present at a given time would not represent the total available reserve carbohydrates of the plant.

4. In an experiment such as this it can readily be seen that dry weight is a wholly unsatisfactory basis for use in calculations, because practically one-fourth of the dry weight is present at a given time as sugars and is presumably nearly all converted to CO_2 and H_2O, which would cause an error of over 30% if the dry basis were used in the calculations.

At the time these two logs were being studied no other log could have been used as an unattacked control without being caged, and the time was not available to run a third log simultaneously. Consequently Tree #7 was not felled until later in the season, when the weather was cooler and no D. brevicomis were in flight. There appears to be little if any significant change in this log, the differences showing no general trend and being hardly larger than the differences between different parts of the same log might be (see Plate 5). This sample contained a large quantity of pitchy substance in the living bark which seemed difficult to remove in clarification. It also gave the highest initial reducing sugar value recorded in the course of this season's work (2.07%).

The fact that the concentrations did not change in this log does not necessarily indicate that changes produced in the attacked trees were entirely due to the beetles directly, or to the organisms which gained entrance with them. The mechanical cutting off of the living portion of the tree into small sections may have been the cause of abnormal physiological activity on the part of the enzymes of the plant itself. The lower temperatures existing after Tree #7 was felled might also account for the lack of change in the sugars.

VI. The relation of growth rate, diameter and tree class to sugar concentrations

Growth rate

One of the primary objects of this season's work was to find out whether any correlation exists between the growth rate of the trees and the chemical composition of the living bark, especially the sugars. Mirov (10) found that the beetles seemed to be attracted to the inner bark, in which they work, more than to other parts of the plant. It had previously been found by Person (15) that a correlation existed between the rate of growth and beetle attack. His studies show that there is a definite selection of the trees of slow growth rate by the western pine beetle.

A series of 11 trees was used in this study, all samples being taken between September 15 and October 15. These trees were not felled, the samples being removed about breast-high on the standing trees. Since the number of trees which could be used in the time available was small, they could not be divided into many classes and still retain a statistically significant group in each class. Consequently three groups were chosen arbitrarily, on the basis of the width of the last complete growth ring. The slow-growing trees were selected as those growing less than 0.5 mm. during the 1928 season; the trees of medium growth rate those whose 1928 ring is from 0.5 to 2.0 mm. wide, and the fast-growing trees those with the annual ring more than 2.0 mm. in width during the same season. This gave four trees in each of the extreme groups and three in the central group, all samples being taken within a month of one another to eliminate as far as possible the effect of seasonal changes. The moisture content, the various sugar values, and the pH were recorded. The average of each of these values for each of the three rates of growth is given in Table VII. The relation of moisture content to growth rate is shown graphically in Plate 6, the relation of some of the sugar values to growth rate in Plate 7, and the relation of pH to growth rate in Pl.8.

The fact that a faster-growing tree has a higher moisture content can be readily observed without analytical methods—in fact, the difference seems greater than here recorded. However, water is of vital importance to the tree, and a small deficiency in actual per cent moisture of trees may be important in areas where moisture is admittedly one of the chief factors limiting growth, as it is in most areas where *brevicomis* is epidemic.

The pH variations do not at first appear to be great and, as will be brought out in connection with Table VIII, are rather inconsistent. However, the total range over which values were found on normal trees in the course of the season was only from 4.3 to 4.6, or 0.3 of a pH division; so a difference of more than 0.1 between the average of the extreme classes would seem relatively large. It is also interesting that the slow-growing trees are more acid, as would be expected if caused by a partial disorganization or fermentation in the cells. In all physiological work it has been found that pH is very important, and more values should be obtained on this point. The data here offered are insufficient as a basis for conclusions.

The differences in sugar concentrations are more definite. The concentration of reducing sugars is larger in the slow-growing trees. This is especially true of levulose, but is also true to some extent of dextrose. The variation in sucrose is in the opposite direction, the highest concentrations being found in the fast-growing trees. This would indicate that as a tree decreases in growth rate a larger share of the sugar is to be found in the simpler forms, as a result of hydrolysis. It will be recalled that this is the same change which took place in the fast-growing tree (FB) after felling. It is highly improbable that this tree would be attacked as a standing tree, but as a trap log it was well attacked. It has been found that fast-growing felled trees are usually attacked

It is not intended to suggest that the actual hydrolysis of sucrose to reducing sugars in either the standing slow-growing tree or the felled log would actually attract beetles. It is a definite possibility, however, that some other change associated with or following this change might produce a volatile substance which would be directly attractive to the beetles. In the cases of both trees of decreasing growth rate and felled trees, the change is from a healthy vigorous cell to one less vigorous and less normally active; that is, it is a change in the direction of death. In this connection it may be to the point to quote Palladin (14)[3].

These changes, produced by enzymes, are largely in the direction of hydrolysis and oxidation, which might account for the shift of the sucrose to its hydrolyzed form, reducing sugars, and the shift of the pH toward the acid side, since the initial oxidation products of sugars are acids.

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[3]Palladin, V.I. - 3rd American Ed. trans. by Livingston: p.172 l. 8-12. When plants are killed without destruction of the enzymes, the physiological system of the cells appears to become completely disarranged, with the destruction of the interrelations that obtain between different constituents of the living cell.

The relation of growth rate to sugar concentration, pH and moisture was also studied on the basis of pairs of trees, one of each pair having low growth rate and the other high. Six pairs of trees were selected, from which the individual samples were taken not over ten days apart and usually on the same day. The results from these pairs of trees were then studied to see whether not only the averages of slow and fast trees were different, but also if there was a consistent trend in one direction rather than a few high or low values. In Table VIII the results are so presented: the number of higher and lower values is given, as is also the number of those not significantly different. Values are considered to be about the same in moisture content if within 0.5% of each other, the same in pH if neither is as much as 0.1 division higher, and alike in sugar concentration if the larger of the pair is not more than 10% larger than the other. The results given here show about the same tendency as that shown in Table VII; but Table VIII brings out the fairly high consistency of the figures on sucrose and moisture, the moderate consistency of the figure on levulose, and the rather inconsistent values on dextrose and reducing sugars and on pH. Both tables indicate a lack of any great difference in total sugars between fast- and slow-growing trees. In order to be certain of the results here obtained a much larger number of pairs would be necessary. The values which show the most positive trends in both methods are moisture content and sucrose, which increase with rate of growth, and levulose, which decreases.

Relation between Dunning's tree classes and moisture content, pH, and sugar concentration

Person (15) found that the amount of loss due to D. brevicomis varied in different tree classes (Dunning's classification), the loss being heaviest in Classes 4 and 5. The data for this season's work were averaged by tree classes to see if any such relationship existed in the substances under investigation. The results are given in Table IX, but Classes 2, 4 and 6 were not represented, and Classes 3 and 7 were represented by only two trees each. Classes 1 and 5 were represented by five dextrose and levulose determinations and by six sucrose, reducing sugar and total sugar determinations. The averages given in Table X show no very pronounced trends. The moisture content of Class 1 trees is slightly higher than the average value, which agrees with the findings in the growth rate studies. The other classes show no significant differences from the average. The pH is not significantly different in any class.

The values for levulose are somewhat lower, and for sucrose somewhat higher, in Classes 1 and 3 (relatively fast-growing) than in Classes 5 and 7 (slow-growing). Dextrose is again uncertain, and total sugars shows practically no differences in different classes.

Relation between diameter and sugar concentration and moisture content

The data in Table XIII were plotted to see whether any relation exists between the diameter of the trees and the sugar concentration. Only a few of the trees used fall within the diameter class found by Person to have the highest beetle losses (24-34"). The averages of the trees of all diameter classes fell very close together. This might be considered to be evidence in support of the theory advanced elsewhere in this report that the relation between sugar concentration and attractiveness to D.brevicomis is indirect rather than direct. The moisture content of the smallest trees (8-18") was slightly higher than the rest (61.35%), but the other averages by 10-inch classes were all close to 60%.

VII. Seasonal Changes

In the course of the season's work it was noticed that the percentage of sugars was increasing. The data for initial determinations given in Table XIII were averaged according to months, and the results shown graphically in Plate 9.

It may be seen from this plate that the main seasonal increase over the period covered by this study is in dextrose. There also seems to be some increase in sucrose, at least in the early part of the season. There does not seem to be any significant seasonal increase in levulose, which is especially interesting, because it is the one sugar which seems to increase definitely with decreased growth rate, i.e., in the same way that attractiveness to D.brevicomis does.

According to the rough means of estimating starch used in the field, it would seem that this substance reached a maximum in midsummer and decreased again in the fall. This does not agree with any carefully-worked-out annual cycles of starch content which could be found in the literature, in that the decrease seemed to occur too early. This method was too inaccurate for the observations here recorded to be very seriously considered. The rather accurate quantitative results obtained in Berkeley agreed with the above in giving a very low value for starch in the late fall.

VIII. Miscellaneous Studies

There were three subjects on which a small amount of work was done to get an idea of the possibilities of the respective fields, but which do not properly fit in elsewhere in this report and so will be included here.

A. Preliminary study on a standing tree attacked by D.brevicomis

A preliminary test was run early in the season on an attacked tree, samples being taken from the attacked and unattacked sides of the base. The attack was fairly good high up but, at the time the samples were taken, it extended only to within reach of the ground on one side. Determinations were run on the inner bark of the attacked and the unattacked sides. The samples were taken when the attack was estimated by Struble to be six days old. The results are shown in Table X. These values (from Tree #4) may be

compared with other initial values in Table XIII. The concentration of sugar is considerably below that of any other tree on which determinations were run during the season. This may have been an initial condition, or it may have been caused by the attack on other portions of the tree. This is the only determination run on a naturally-attacked standing tree. Other such determinations should be made and followed through, as the changes on trap logs were this season. It was impossible to do this in 1929, due to lack of time and scarcity of summer brood trees in the vicinity of the field laboratory.

D. Determinations on outer bark

When the parent adult beetles enter a tree during attack, they must enter through the outer dead bark, and the larvae ordinarily enter this layer when about one-quarter grown and complete their development and pupate in it. The new adults then bore on out to the surface. From this it may be seen that the outer bark enters very closely into the life of the beetle. However, in the attraction studies previously referred to, it was not found as attractive as the inner bark; and since it is not living, it would not be subject to change in composition and consequent change in attractiveness, as the tree has been shown to be. Determinations similar to those conducted on the inner bark of the other trees were conducted on the outer bark of the three trees most completely studied, i.e., Nos. 5,6 and 7. The results are given in Table XI, where they are compared with the initial and final values on the inner bark. There is a possibility that some substance other than sugar, such as tannin, may be present in the outer bark in a much higher concentration than in the inner bark and so make those values too high; but it is unlikely that non-sugars account for a very large fraction of the material reported as sugar. The values found for sugars in the outer bark are lower than the original sugar concentrations in the inner bark, but higher than the final concentrations in the cases (5 and 6) where the trap logs were attacked by D. brevicomis. By referring back to Tables V and VI it may be seen further that by the time the larvae deserted the inner bark it had already dropped below the outer bark in sugar concentrations. Consequently the migration of the quarter-grown larvae to the outer bark may be at least partly due to the deficiency of sugars in the inner bark.

It may be seen that the death of the cells of the inner bark, either by the attack of felled trees or by the cutting off of the cells by the development of a new phellogen, results finally in a decided loss in sucrose and levulose without a corresponding reduction in dextrose. The disappearance, however, is not so complete as in natural death, as in the case where microorganisms were present, also. The pH is much lower; i.e., the acidity is much higher, in the dead outer bark than in the inner bark after attack and yeast and "blue stain" development.

E. Fire injury

Determinations were run on one tree (21) to obtain an indication of the effect of fire injury on sugar concentrations. This was a large, healthy, Class III tree of medium growth rate, and not of a type normally attacked. Seventy-five per cent of the needles had been scorched in the Sugar Hill fire of July 24, but the cambium did not appear to be abnormal;

in fact, this year's growth ring was wider than those of previous years. (Growth 1929 0.83 mm., 1928 0.72, 1927 0.82, 1926 0.80). The sample was taken October 15, 81 days after the fire.

The results can best be observed in connection with the rest of the initial values in Table XIII. The values obtained for it are considerably different from the values from other trees sampled at about the same date, but are similar to those sampled about the date on which the area around this tree was burned over (July 24). When the individual variation between trees is as great as was found in these studies, the results from one tree cannot be accepted, however, as more than an indication.

It has been shown by Miller (11) that after fire has injured trees such as this, the amount of insect loss increases enormously--from 0.9% in the uninjured trees to 78.9% in trees of Burn Class IV, of which 75% of the crown was defoliated. The tree here used would belong to this class, but would be larger than the average of the members of this class used in the burn study.

It would be very interesting to have similar determinations on this and other trees during the 1930 season, when the insect loss in the burned-over area will probably be much greater and the chance of attack of these trees large. It may be of importance that the pH value obtained on this tree was 4.7, which is higher than that of any other tree studied.

IX. Sugar and starch determinations at Berkeley

Conditions at the Buck Creek Field Laboratory could not be made in every way similar to conditions in the laboratories where the methods used were worked out. The most important difference in conditions is the boiling point of water, as influenced by the elevation. The boiling point at the field laboratory is about 95° C. (203° F.). Since the basic determination depends on boiling the solution for a given time, results would not be expected to be the same as at sea level, because the rate of a chemical reaction increases considerably with temperature. As before noted, the factor found for the method was the same as that found by the original authors, but it was still thought advisable to get determinations at Buck Creek and Berkeley as nearly comparable as possible.

Determinations were run on Tree #22 shortly before leaving the field laboratory, and the tree was felled and a log removed just before departure. This log was placed in cold storage at 5° C. (41° F.) upon arrival at Berkeley two days later. Four days later another sample was taken from the log and sugar determinations made. These results are given in Table XII, in comparison with the values obtained at the field laboratory and those obtained after prolonged cold storage. The moisture content was found to be smaller in the determinations after arrival at Berkeley. These latter determinations were made in a vacuum oven at about 60 mm. Hg and 73° C. (163° F.) instead of a water oven under atmospheric pressure at 94° C. Levulose starts to decompose at 80° C., which tends to give too high a value for moisture, as does also the loss of volatile substances other than water. This latter loss is also greater at the higher temperature. The vacuum oven gives more accurate results than the water oven.

but requires electricity for heating and evacuation. There was a slight difference in the recorded pH, which is undoubtedly within the limit of experimental error. The results on sugars are very similar for the reducing sugar and levulose determinations, but the sucrose value is very much lower.

The only explanation that can be suggested for this change is on the basis of two simultaneous processes. One of these would be an hydrolysis of sucrose to reducing sugar, and the other a disappearance of reducing sugar by some form of respiration. There is no evidence, however, of a production of moisture, so the respiration, if any, would have to be abnormal. The results obtained later, after prolonged cold storage, fit in fairly well with this suggestion. It does not seem possible that this difference can be due to the difference between conditions at the two laboratories, as it is in the opposite direction from difference due to boiling point.

Three months later another sample was run to find what further changes has taken place during prolonged cold storage. The results are included in the last column of Table XII. The sucrose has decreased a little more and the dextrose has fallen significantly. Levulose has again risen slightly. The total sugars have fallen, due to the loss of sucrose and dextrose. The changes taking place are small, however, compared to those between the first two columns. This is to be expected, because at the time the log was transported to Berkeley the temperature was rather high—80-90° F. This was followed by a sudden change to 41° F. Probably the greatest change in condition in this latter period is a very considerable loss of moisture. This would probably not have been so great if the cold storage room had remained unopened during this time, but it was in constant use. There does not seem to be any significant change in pH during the whole experiment. The results on this log and on the next would seem to indicate clearly that experiments similar to these could not be run with any degree of accuracy unless the work was done in a laboratory close to the trees being studied, and the material handled as quickly as possible.

Starch determinations

The quantitative starch determinations were conducted on log #22 used in the foregoing section. Samples of inner bark were dried in the vacuum oven at 70° C. and in the oven at atmospheric pressure regulated to 105° C. (221° F.). The results on the former averaged 2.70% starch and on the latter 3.56%. These values are much lower than the summer values, and probably somewhere near the value at which it would remain for the winter.

A log (#25) was also brought in from near Northfork, Calif., on the Sierra National Forest, in the same manner as #22 was brought from Buck Creek. The results obtained on this log are given in Table XIII with the rest of the initial values. It will be seen that the results for both the reducing sugars, levulose and dextrose, are considerably higher than the average figures obtained during the summer; in fact, levulose was present in the highest concentration obtained on any tree. This is particularly noticeable because levulose was ordinarily low on fast-growing trees, such as this one. Sucrose, on the other hand, was present in the lowest concentration found, where it would be expected to be high, as in other fast-

growing trees. These differences are probably due to changes in the log after felling, resembling those found in the log similarly brought from the field laboratory at Buck Creek.

The changes in these two logs, Nos. 22 and 25, were rather violent, but they were both cut out of the felled trees and transported for considerable distances under temperature conditions which would favor rapid change. It may be seen, however, by referring to Plate 2 (Tree #5), that similar changes had started on this trap tree before it could possibly have been affected by insect attack; i.e., in the period during which it was becoming attractive. This may be very important when considered in connection with the increase in attractiveness of standing trees as they increase in growth rate. The direction of change in sugar concentration is the same in both cases—i.e., an increase in levulose and a decrease in sucrose.

X. <u>Suggestions for future work</u>

The work reported in this paper is in a field in which very little has been done; consequently much of the time during the past field season was spent in very preliminary work on methods and technique. However, it would appear that several interesting leads have been developed. The changes in attacked trap logs were brought to a more definite status than any other work. The correlation between these changes and the conditions in slow-growing trees offers a very interesting and possibly important line for further work. A great deal more should be done on the changes which take place in attacked trees. Another place where additional work is almost certain to provide interesting data is in the study of the changes which accompany injury of the types known to increase the likelihood of <u>D.brevicomis</u> attack, such as drought, defoliation and fire injury.

Another section of this study which should be amplified is the determination of pH. After practice, fairly good comparative results could be obtained by direct staining of the inner bark or other portions of the tree. By taking data of this kind on one of the sample plots already being maintained and on which the individual tree records have been taken, results on a large number of trees would be available with a comparatively small amount of labor.

The small number of trees used is probably the greatest source of error in the work here reported; but is an error that is almost certain to appear where the determining of a single set of results takes as much time as it does in all known methods of sugar analysis.

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Table I

Changes in sugar concentration (% wet basis) on short logs

Tree Number	Growth Rate	Date	Reducing Sugar	Sucrose	Total
8	Slow	6/ 4	1.86	1.90	3.76
		6/10	1.75	2.18	3.93
		6/27	1.16	1.10	2.26
5	Fast	6/13	1.64	2.54	4.18
		6/29	2.83	1.55	4.38

Table II

Changes in the sugar concentration of the inner bark of Log #2

Sugars	Initial	Top Unattacked	Side Attacked
Reducing sugars	1.64	1.66	5.25
Levulose	—	1.33	2.41
Dextrose	—	0.33	2.84
Sucrose	2.54	1.16	1.77
Total sugars	4.18	2.82	7.02

<u>Table III</u>

Changes in the sugar concentrations - log 10

Sugars	Initial	Top (egg stage)	Side (larvae leaving inner bark)
Reducing	1.85	0.94	0.26
Levulose	—	0.60	0.25
Dextrose	—	0.34	0.01
Sucrose	1.90	0.36	0.19
Total	3.75	1.30	0.45

Table IV

Changes in Sugar Concentration of a Fast-Growing Tree (#3) After Felling and Subsequent Attack

Date	Sample No.	Days Felled	% Moisture	Per Cent Sugar on Fresh Weight					Per Cent Sugar on Dry Weight					Remarks
				Red.	Lev.	Dext.	Suc.	Total	Red.	Lev.	Dext.	Suc.	Total	
7/25	1	-4	62.3	1.71	1.16	0.68	4.38	6.03	4.53	3.08	1.45	11.46	15.99	SE side
7/25	2	-4	60.5	1.38	1.25	0.63	4.04	5.92	4.85	3.16	1.60	10.22	14.97	NE "
7/29	3	0	63.7	1.44	0.83	0.56	4.71	6.13	3.97	2.39	1.53	10.84	15.49	
7/29	4	0	63.7	1.50	0.89	0.61	4.88	6.38	4.13	2.42	1.71	12.98	16.95	Tree felled
7/30	5	1	62.45	1.67	1.07	0.60	3.63	5.35	4.44	2.85	1.59	9.81	14.25	
8/1	6	3	62.25	1.98	1.20	0.78	4.60	6.58	5.24	3.18	2.06	12.18	17.42	
8/5	7	7	61.4	2.54	1.43	1.11	4.14	6.68	6.59	3.71	2.87	10.73	17.31	
8/5	8	7	60.6	2.60	1.58	1.02	4.06	6.66	6.60	4.01	2.59	10.31	16.91	Attack Started
8/13	9	15	58.0	4.96	3.50	1.45	2.63	7.59	11.78	8.33	3.45	6.26	18.04	
8/19	10	21	57.5	3.86	2.74	1.12	2.17	6.03	9.08	6.44	2.64	5.11	14.19	Unattacked
8/19	11	21	57.5	6.42	3.88	2.54	3.70	10.12	15.11	9.13	5.98	8.70	23.81	Attacked
8/28	12	30	61.04	2.13	1.57	0.56	2.08	4.21	5.44	3.97	1.47	5.22	10.66	Unattacked
8/28	13	30	57.1	1.75	1.34	0.41	0.88	2.63	4.03	3.12	0.95	2.05	6.13	Attacked
9/12	14	45	59.9	0.95	0.34	0.61	0.28	1.23	2.37	0.85	1.52	0.70	3.07	Attacked
10/7	15	70	67.3	0.30	-0.05	0.30	0.15	0.45	0.92	-0.15	0.92	0.46	1.38	Attacked
10/7	16	70	15.9	1.13	0.23	0.98	0.05	1.34	1.42	0.27	1.15	0.77	2.19	Outer Bark
10/9	17	72	67.9	0.31	0.01	0.30	0.04	0.35	0.97	0.03	0.94	0.12	1.09	Inner Bark

Table V

Changes in Sugar Concentration of a Slow-Growing Tree (#6) After Felling and Subsequent Attack

Date	Sample No.	Days Felled	% Moisture	Fresh Weight Red.	Fresh Weight Lev.	Fresh Weight Dext.	Fresh Weight Suc.	Fresh Weight Total	Dry Weight Red.	Dry Weight Lev.	Dry Weight Dext.	Dry Weight Suc.	Dry Weight Total	Remarks
8/7	1	0	57.4	2.85	1.09	1.86	3.04	6.49	6.73	3.75	4.90	8.59	15.32	Outside-fld.
8/7	2	0	54.4	3.04	1.65	1.39	3.17	6.21	6.66	3.62	3.04	6.95	13.61	[illegible]
8/13	3	6	52.85	5.09	3.30	1.79	2.90	7.99	10.75	6.97	3.78	6.12	16.87	Attacked
8/20	4	13	55.3	1.30	0.94	0.36	0.69	1.99	2.95	2.15	0.82	1.54	4.51	"
8/26	5	19	54.1	0.33	0.04	0.29	0.34	0.67	0.72	0.09	0.63	0.74	1.46	"
8/26	6	19	6.9	0.84	0.16	0.68	0.31	1.15	0.90	0.17	0.73	0.33	1.23	Out.Bark
9/12	7	36	54.4	0.03	-0.01	0.33	0.57	0.60	0.07	-0.02	0.07	1.25	1.31	Inn.

Table VI

Changes in Sugar Concentration of a Slow-Growing Tree (#7) After Felling

Date	Sample No.	Days Felled	% Moisture	Fresh Weight Red.	Fresh Weight Lev.	Fresh Weight Dext.	Fresh Weight Suc.	Fresh Weight Total	Dry Weight Red.	Dry Weight Lev.	Dry Weight Dext.	Dry Weight Suc.	Dry Weight Total	Remarks
9/4	1	0	57.45	5.07	3.11	2.96	2.61	7.68	11.91	4.98	6.95	6.14	18.05	Tree fld.
9/4	2	0	5.22	3.80	1.12	2.67	0.98	4.78	4.01	1.19	2.82	1.03	5.04	Out.Bark
9/9	3	5	55.15	3.39	2.07	1.92	3.85	7.24	8.50	4.61	4.29	7.85	16.15	Inn.
9/9	4	5	4.33	2.52	1.01	1.51	0.77	3.29	2.63	1.06	1.61	0.82	3.81	Out.
9/15	5	14	55.6	4.68	2.33	2.43	3.23	7.91	10.10	4.28	5.52	6.97	17.07	Inn.
9/19	6	16	54.3	4.71	2.59	2.12	2.96	7.67	10.29	5.65	4.63	6.46	16.74	"
9/30	7	26	51.22	4.71	2.49	2.22	3.65	8.36	9.66	5.10	4.56	7.54	17.20	"
10/13	8	39	52.75	4.54	2.10	2.36	2.29	6.83	9.61	4.61	5.00	4.83	14.44	"

Table VII

Variation of average per cent moisture, pH, and per cent sugars with growth rate

Growth Rate	Slow	Medium	Fast
Width of 1938 ring mm.	0.0 - 0.5	0.5 - 2.0	2.0+
Moisture	58.63	61.30	68.90
pH	4.43	4.50	4.55
Sugars			
Reducing	3.55	2.97	2.74
Levulose	1.84	1.30	1.26
Dextrose	1.71	1.67	1.48
Sucrose	3.03	4.29	4.35
Total Sugars	6.58	7.26	7.09

Table VIII

Growth rate vs. moisture content, pH and sugar concentration; consistency of results

	Slow Higher	Fast Higher	About the same
Moisture	1	4	1
pH	2	3	1
Sugars			
Reducing	3	2	1
Levulose	2	1	2
Dextrose	3	2	1
Sucrose	1	4	1
Total Sugars	0	2	4

Table IX

Dunning's tree classes vs. moisture content, pH and sugar concentrations

Class	1		3		5		7		Average
	Value	Per cent of Average	Value	Per cent of Average	Value	Per cent of Average	Value	Per cent of Average	
Moisture	62.50	103.0	59.59	98.6	59.95	98.8	58.47	96.4	60.65
pH	4.35	100.8	4.45	99.0	4.[illegible]	100.8	4.50	100.0	4.50
Sugars									
Reducing	2.74	92.6	2.44	82.2	3.06	103.3	3.84	129.9	2.96
Levulose	1.33	87.5	1.31	86.2	1.65	108.5	1.86	122.4	1.52
Dextrose	1.63	101.2	1.15	71.0	1.65	101.9	1.98	122.1	1.62
Sucrose	3.97	111.2	4.43	124.1	3.07	86.1	2.97	83.2	3.57
Total	6.71	102.8	6.89	105.3	6.13	93.9	6.81	104.1	6.53

Table X

Attacked Tree 4 (14-2)

Sugar	Unattacked		Attacked	
	Fresh wt.	Dry Wt.	Fresh Wt.	Dry Wt.
Reducing	1.35%	3.63%	0.61%	1.63%
Levulose	0.77	2.07	0.39	1.04
Dextrose	0.58	1.56	0.22	0.59
Sucrose	1.65	4.44	—	—
Total	3.00	8.07	—	—
Moisture	62.8		62.6	

Table XI

Determinations on outer bark

Portion of bark sampled	Tree Number 5 Initial	5 Final	5 Outer	6 Initial	6 Final	6 Outer	7 Initial	7 Final	7 Outer
% moisture	61.4	67.6	15.9	56.9	54.9	6.9	56.50	52.75	5.70
pH	4.4	4.4	5.0	4.6	4.6	5.0	4.8	4.5	5.0
Sugar determinations									
(wet basis)									
Reducing	1.80	0.30	1.19	2.95	0.03	0.34	4.53	4.54	3.16
Levulose	1.21	0.02	0.23	1.63	0.01	0.16	2.09	2.18	1.07
Dextrose	0.59	0.30	0.96	1.32	0.03	0.65	2.44	2.36	2.09
Sucrose	4.18	0.10	0.65	3.40	0.57	0.31	2.93	2.28	0.88
Total Sugars	5.98	0.40	1.84	6.35	0.60	1.15	7.46	6.82	4.04
(dry basis)									
Reducing	4.69	0.94	1.42	6.70	0.09	0.90	10.41	9.01	3.35
Levulose	3.12	0.06	0.27	3.69	0.02	0.17	4.79	4.01	1.14
Dextrose	1.57	0.84	1.15	3.01	0.07	0.73	5.62	5.00	2.21
Sucrose	10.84	0.29	0.77	7.77	1.22	0.33	6.69	4.23	0.92
Total Sugars	15.43	1.23	2.19	14.47	1.31	1.23	17.10	14.44	4.27

Table XII

Comparison of the values on Tree 22 obtained at the Buck Creek Field Laboratory, after transportation of a log to Berkeley, and after prolonged cold storage

	Buck Creek Field Laboratory	Berkeley Initial	Berkeley After cold storage
Per cent Moisture	60.7	59.5	58.1
pH	4.4	4.45	4.4
Sugar determinations (wet basis)			
Reducing	3.84	3.93	3.38
Levulose	1.62	1.72	1.79
Dextrose	2.22	2.21	1.59
Sucrose	3.90	2.20	2.03
Total	7.74	6.13	5.41
(dry basis)			
Reducing	9.77	9.67	7.53
Levulose	4.12	4.23	3.99
Dextrose	5.65	5.44	3.54
Sucrose	9.92	5.41	4.52
Total	19.69	15.08	12.05

Table XIII

Initial Determinations on Trees - Wet Basis

Date	Tree Number	Running Class	DBH	Rings last ½"	Ring Width mm.	% Moisture	pH	Per cent Sugar on Fresh Weight: Red.	Lev.	Dex.	Suc.	Tot.	Per cent Sugar on Dry Weight: Red.	Lev.	Dex.	Suc.	Tot.	Remarks
6/ 3	0	-	-	-	-	-	-	1.74	-	-	-	-	-	-	-	-	-	Experiments
6/ 3	1	-	-	-	-	-	-	1.36	-	-	-	-	-	-	-	-	-	on Method
6/ 4	2	5	24	65	-	-	-	1.65	-	-	1.90	3.75	-	-	-	-	-	Deterioration
6/13	3	1	20	5	3.91	-	-	1.64	-	-	2.54	4.18	-	-	-	-	-	Studies
7/16	4	5	-	-	-	62.8	-	1.36	0.77	0.58	1.66	3.00	3.67	2.07	1.56	4.44	8.07	Attached above by D.b., same as B-2, Buck Creek Cruise
7/28	5	3	28	7	1.45	61.4	4.4	1.80	1.21	0.59	4.18	5.98	4.66	3.14	1.53	10.82	16.48	Changes
8/ 7	6	7	20	39	0.27	55.9	4.6	2.95	1.62	1.55	3.41	6.36	6.70	3.68	3.02	7.74	14.44	after
8/ 4	7	7	18	27	0.27	37.45	4.6	3.07	2.11	2.96	2.61	7.68	11.90	4.95	6.95	6.13	18.98	Felling, etc.
9/16	11	1	16	2	5.56	63.6	4.6	2.03	1.00	1.03	4.65	6.68	5.56	2.75	2.82	12.78	18.36	
9/18	12	1	14	4	3.97	61.5	4.5	3.19	1.45	1.73	4.00	9.18	8.26	3.77	4.49	15.58	23.85	Growth
9/25	13	7	22	35	0.10	59.5	4.4	2.61	1.60	1.01	3.33	5.94	6.45	3.95	2.50	8.23	14.68	
9/23	14	1	23	8	2.07	63.1	4.6	2.93	1.50	1.43	3.70	6.63	7.98	4.07	2.88	10.02	17.97	
9/28	15	5	48	19	0.73	64.25	4.6	1.97	0.88	1.09	4.30	6.27	5.61	2.50	3.11	12.22	17.83	Rate
9/28	16	1	8	3	4.25	63.4	4.5	2.94	1.10	1.74	3.04	5.88	7.76	3.01	4.75	8.31	16.07	
9/30	17	5	54	44	0.10	60.85	-	3.90	2.28	1.62	2.43	6.33	10.00	5.62	4.18	6.34	16.34	Studies
10/ 2	18	5	42	54	0.11	57.6	4.5	3.18	1.46	1.72	2.78	5.96	7.51	3.45	4.06	6.56	14.07	
10/ 2	19	3	41	10	1.47	58.35	4.5	3.11	1.41	1.70	4.68	7.79	7.46	3.38	4.08	11.21	18.67	etc.
10/ 9	20	5	34	23	0.36	60.55	4.6	4.49	2.01	2.42	3.55	8.04	11.30	5.09	6.30	9.01	20.40	
10/13	21	3	24	17	0.72	60.7	4.7	2.95	1.65	1.32	2.10	5.05	7.30	4.15	3.35	5.34	12.34	75 Defol. by Fire
10/15	22	1	14	6	1.56	60.7	4.4	3.54	1.62	2.22	3.99	7.74	9.77	4.12	5.65	9.92	19.69	Gr. Rate Stud., etc.
11/ 5	23	1	12	4	1.54	62.0	-	4.90	2.81	2.09	1.63	6.53	12.30	7.39	5.30	4.29	17.13	Sample from North-fork, Calif.

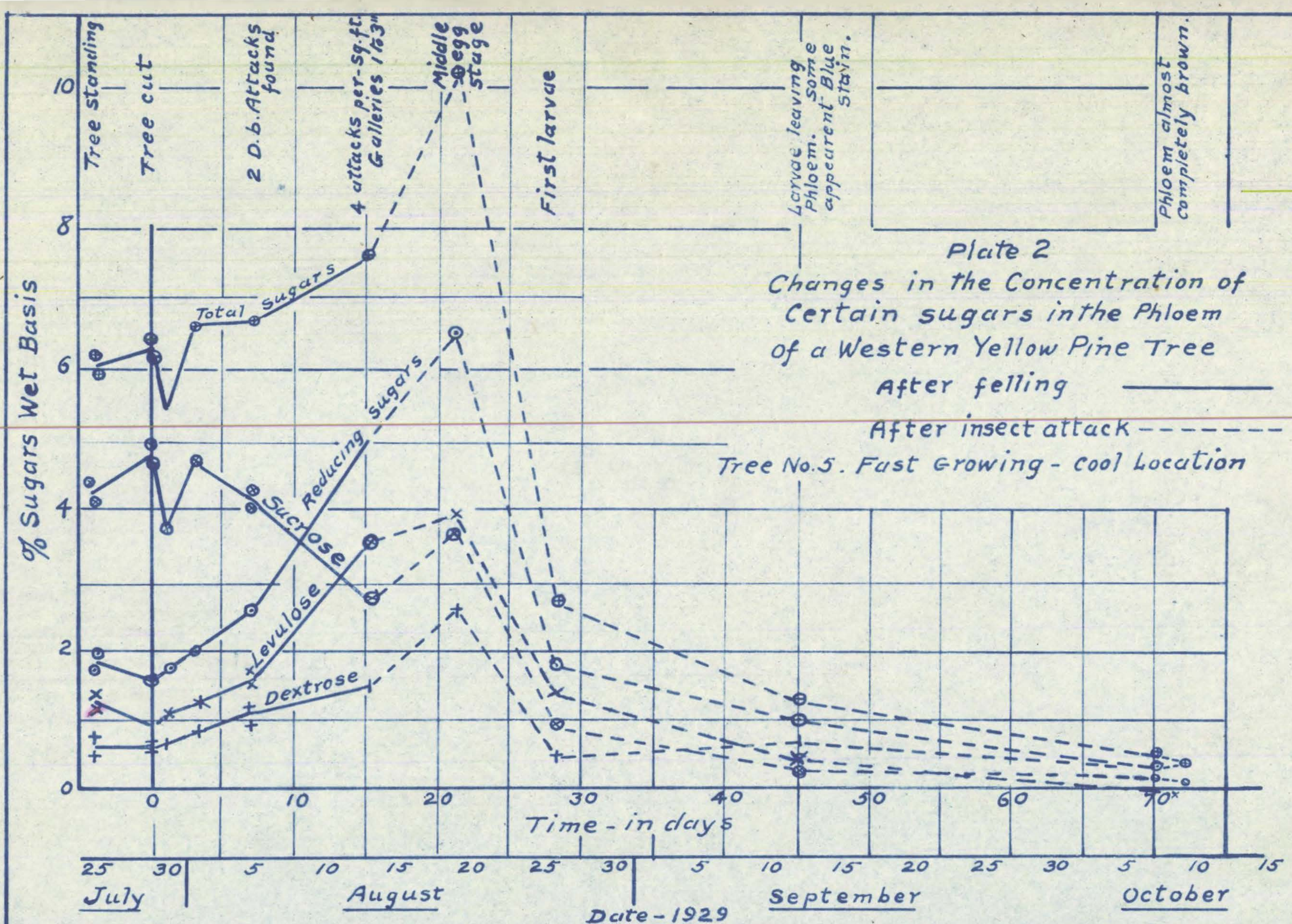


Plate 2

Changes in the Concentration of Certain sugars in the Phloem of a Western Yellow Pine Tree

After felling ————

After insect attack - - - - - -

Tree No. 5. Fast Growing - Cool Location

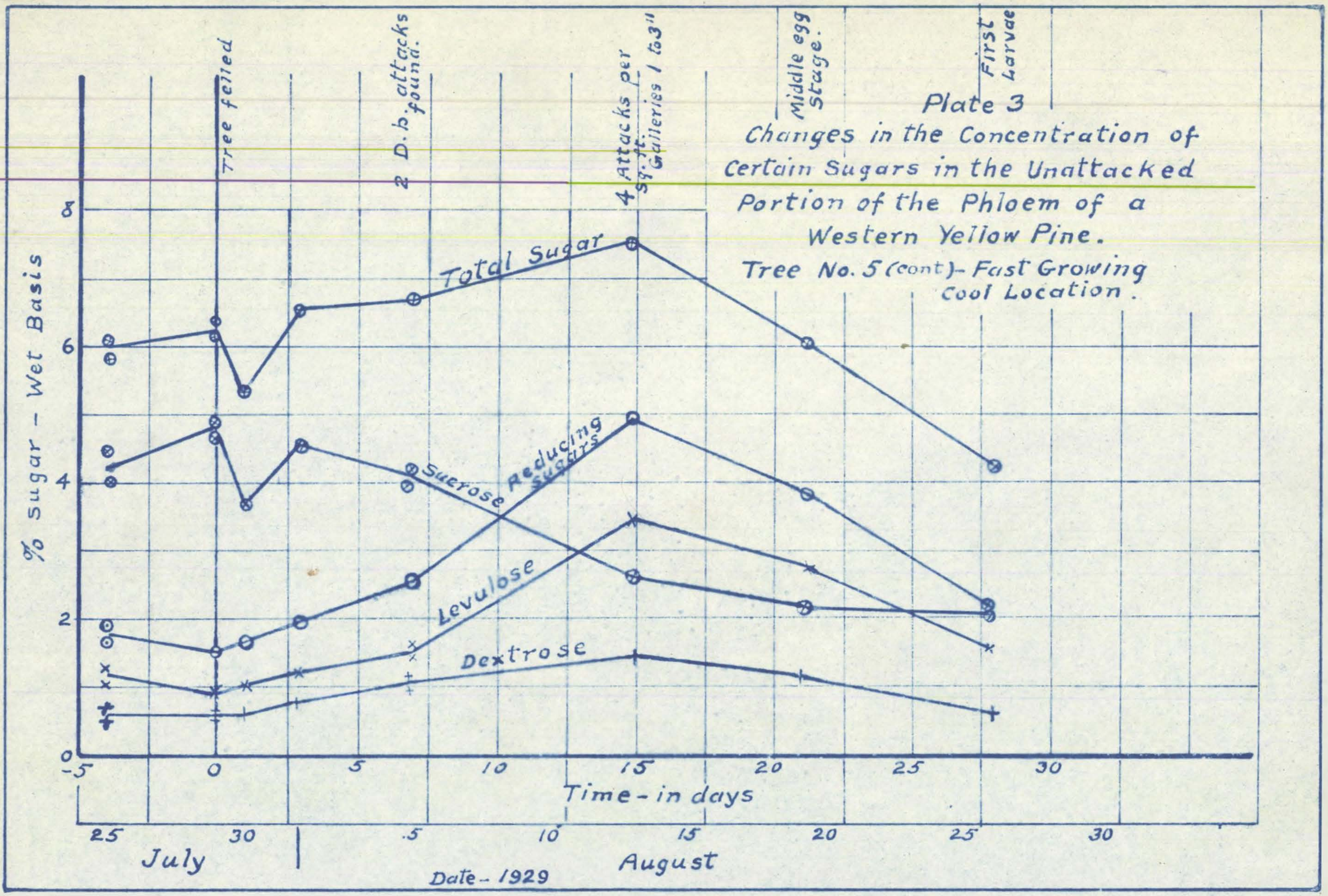
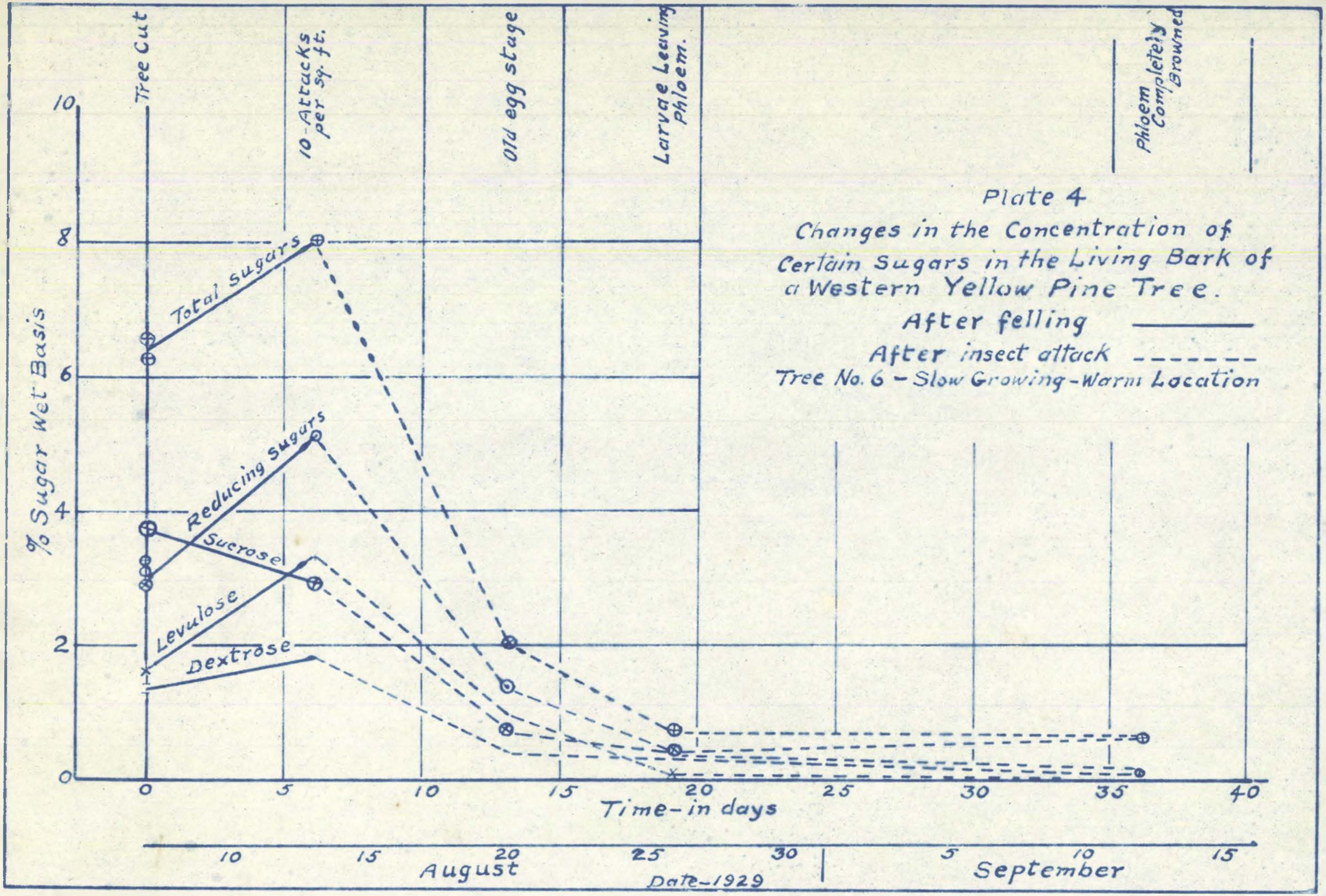


Plate 3
Changes in the Concentration of Certain Sugars in the Unattacked Portion of the Phloem of a Western Yellow Pine.
Tree No. 5 (cont) - Fast Growing Cool Location.
Tree felled
2 D.b. attacks found.
4 Attacks per sq. ft. Galleries 1 to 3"
Middle egg Stage.
First Larvae
Total Sugar
Sucrose
Reducing Sugars
Levulose
Dextrose
% Sugar - Wet Basis
0
2
4
6
8
-5
0
5
10
15
20
25
30
Time - in days
25
30
July
.5
10
15
20
25
30
August
Date - 1929



Tree Cut
10-Attacks per sq. ft.
Old egg stage
Larvae Leaving Phloem.
Phloem Completely Browned
Plate 4
Changes in the Concentration of Certain Sugars in the Living Bark of a Western Yellow Pine Tree.
After felling
After insect attack
Tree No. 6 - Slow Growing - Warm Location
Total Sugars
Reducing Sugars
Sucrose
Levulose
Dextrose
% Sugar Wet Basis
0
2
4
6
8
10
0
5
10
15
20
25
30
35
40
Time - in days
10
15
20
25
30
5
10
15
August
September
Date - 1929

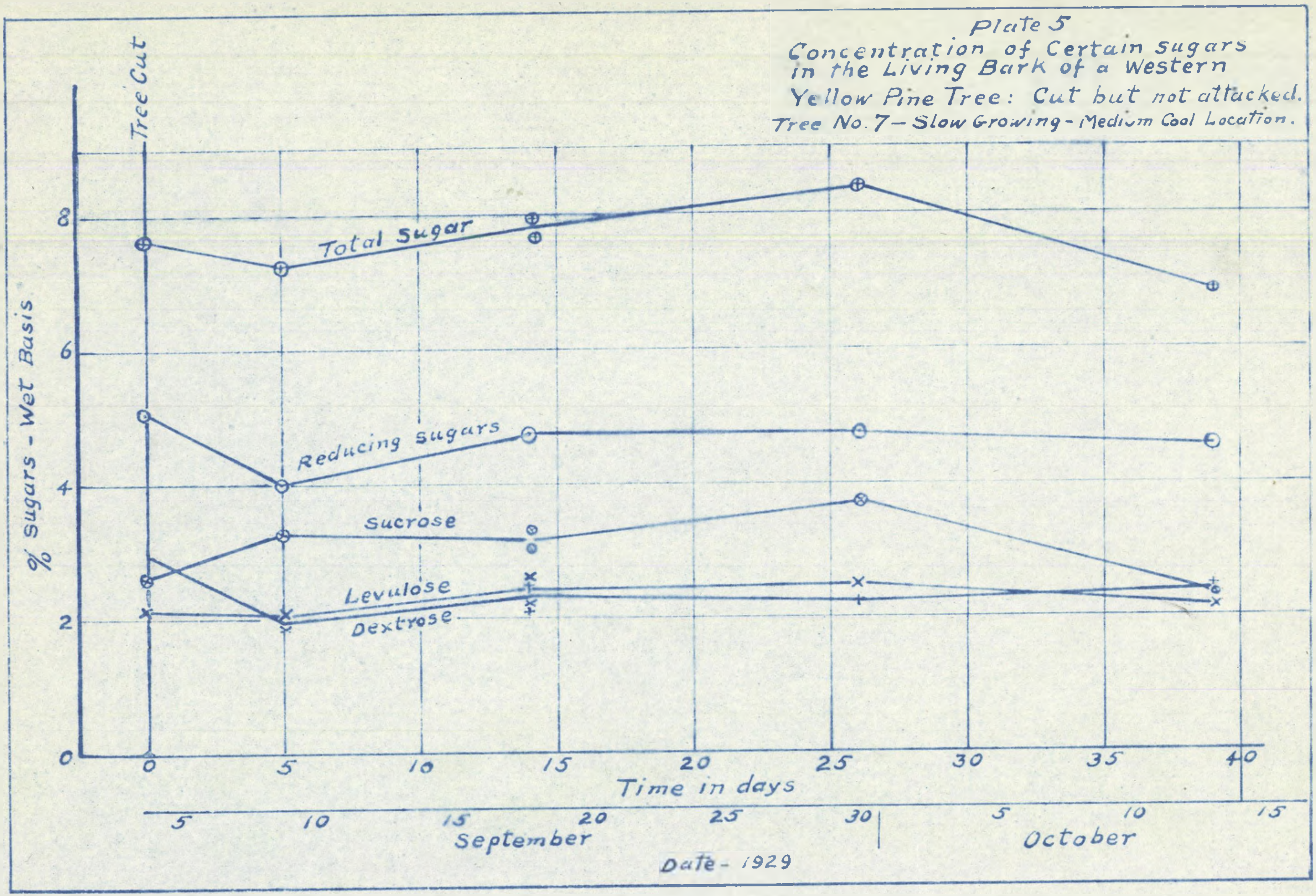


Plate 5
Concentration of Certain sugars in the Living Bark of a Western Yellow Pine Tree: Cut but not attacked.
Tree No. 7 - Slow Growing - Medium Cool Location.
Tree Cut
% Sugars - Wet Basis
Total Sugar
Reducing sugars
Sucrose
Levulose
Dextrose
0
2
4
6
8
0
5
10
15
20
25
30
35
40
Time in days
5
10
15
20
25
30
5
10
15
September
October
Date - 1929

Plate 6

Relation between Growth Rate and the Percentages of Moisture in the Living Bark of Western Yellow Pine

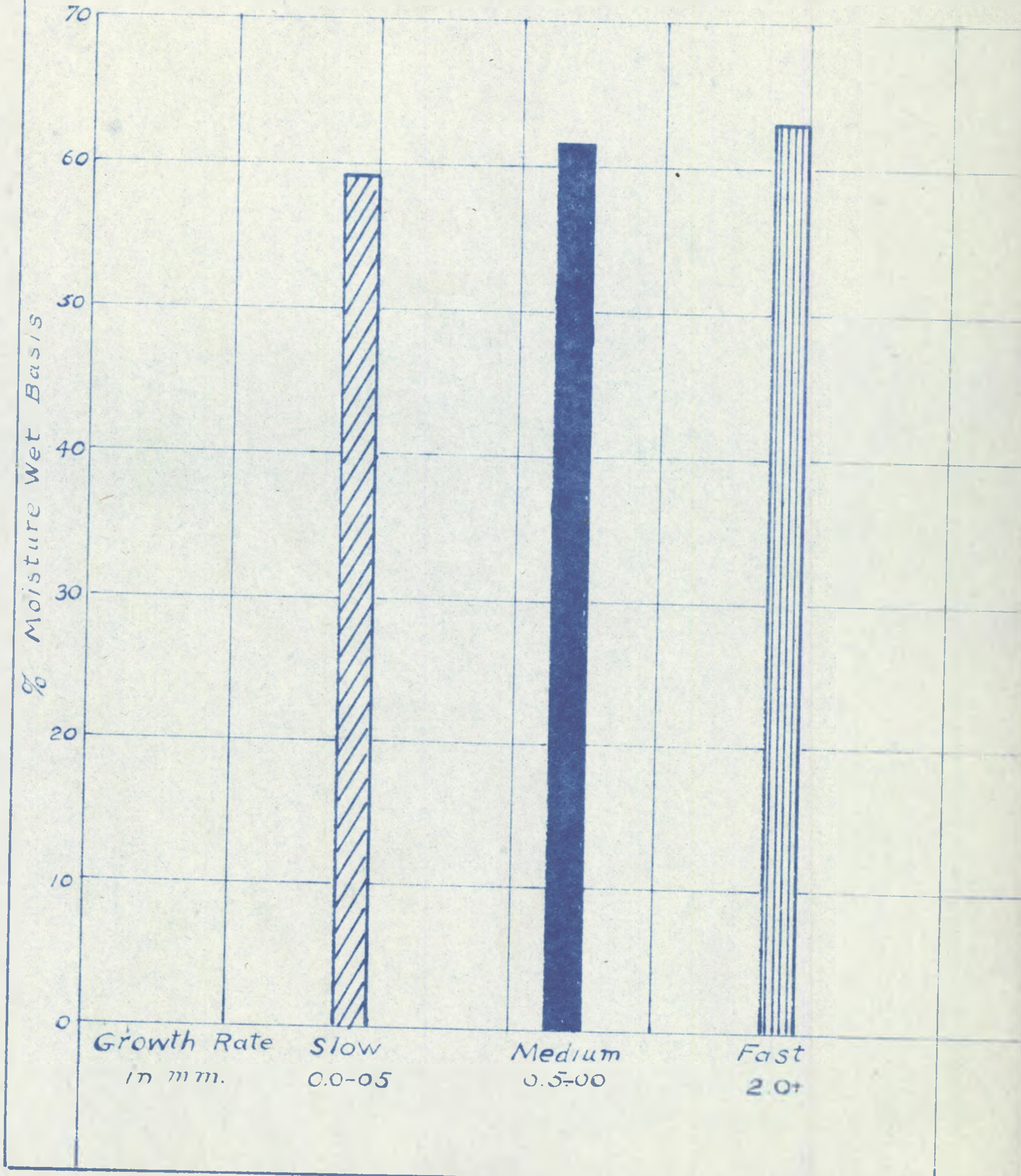
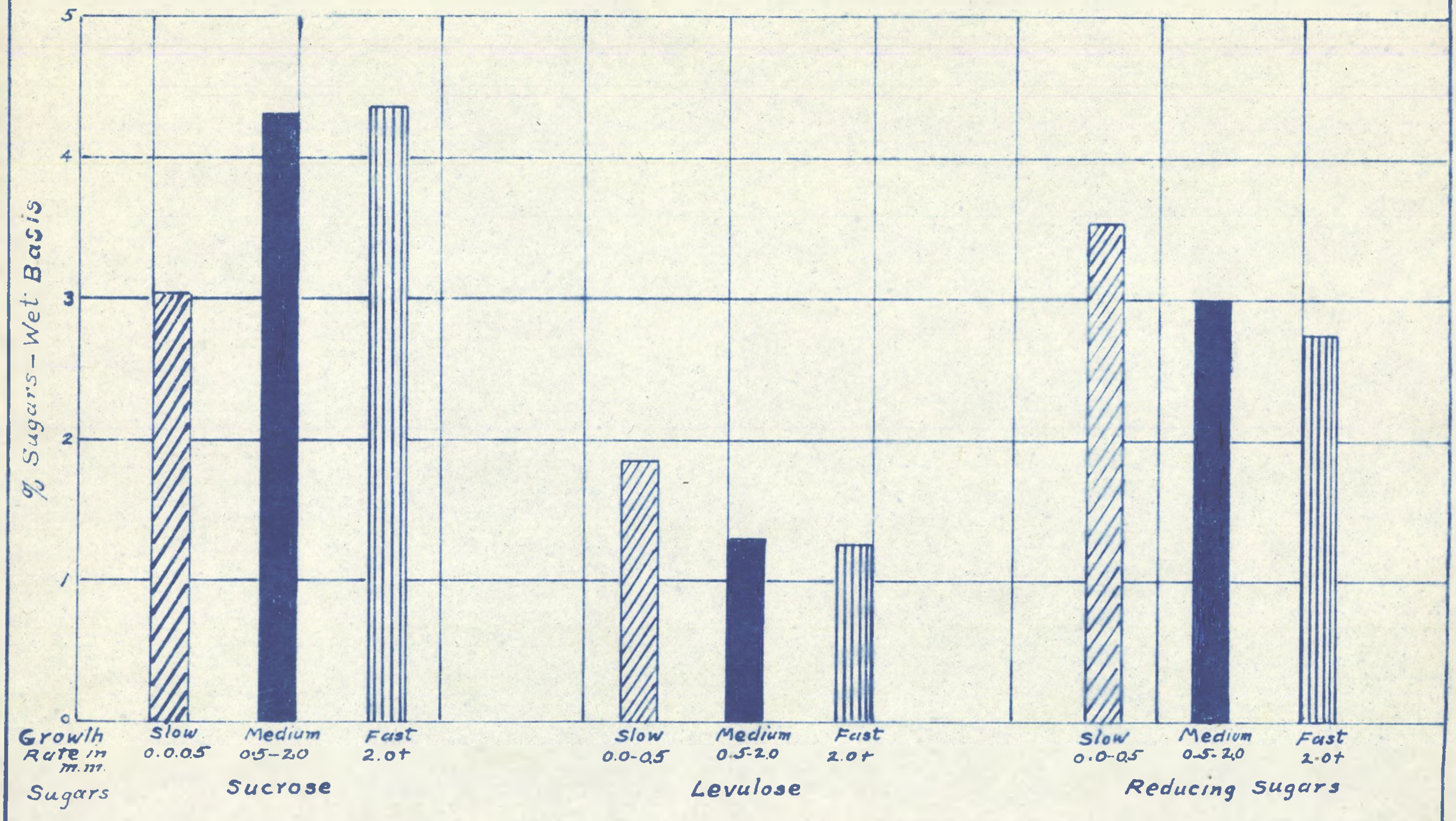


Plate 7
Relation Between Growth Rate and the Concentration of Certain Sugars in the Living Bark of Western Yellow Pine.



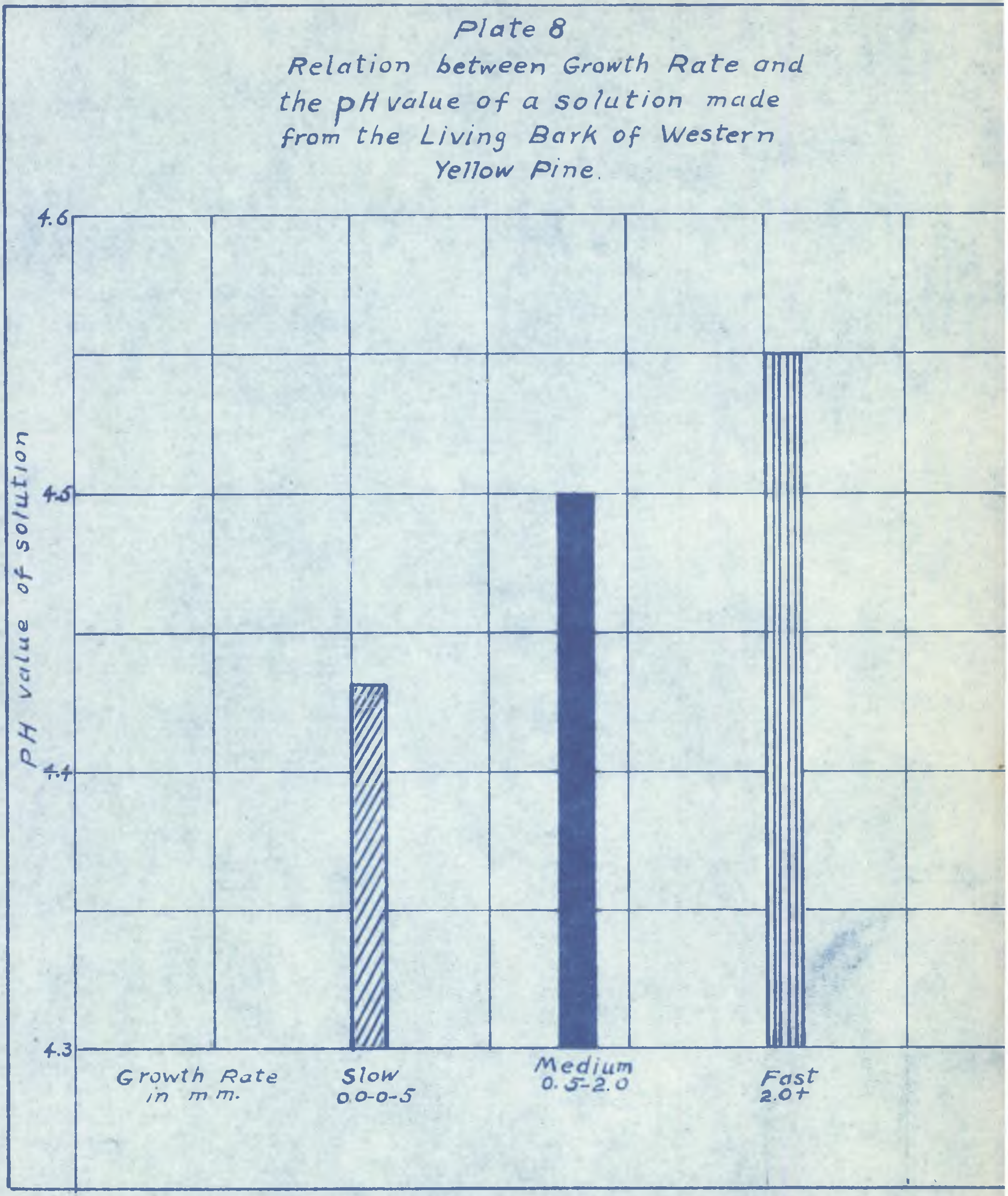


Plate 8
Relation between Growth Rate and the pH value of a solution made from the Living Bark of Western Yellow Pine.
4.6
4.5
4.4
4.3
PH value of solution
Growth Rate
in mm.
Slow
0.0-0-5
Medium
0.5-2.0
Fast
2.0+

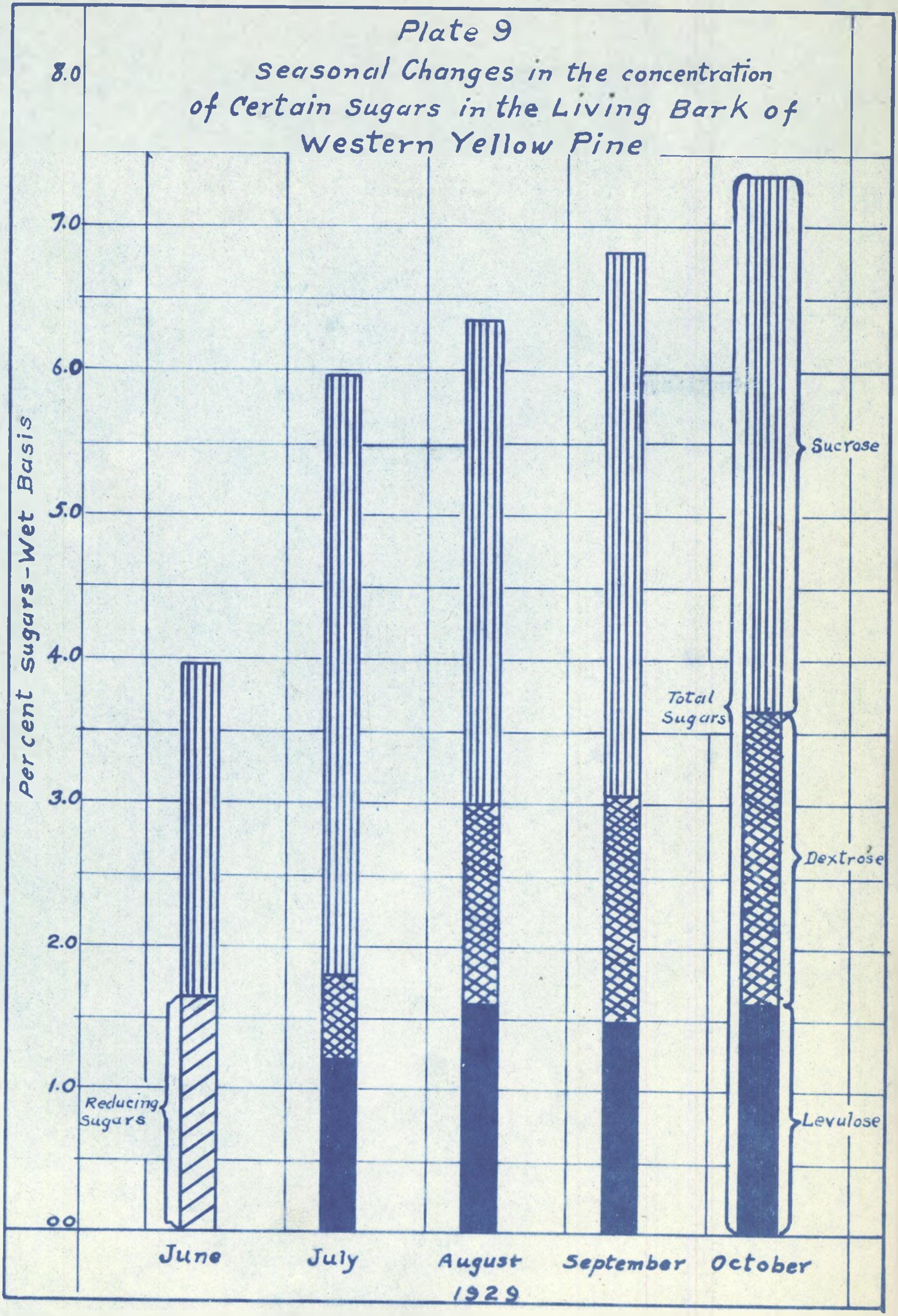


Plate 9
Seasonal Changes in the concentration of Certain Sugars in the Living Bark of Western Yellow Pine
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Percent Sugars-Wet Basis
Sucrose
Total Sugars
Dextrose
Levulose
Reducing Sugars
June
July
August
September
October
1929